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Pea cultivar and wheat residues affect carbon/nitrogen dynamics in pea-triticale intercropping: a microcosms approach

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Abstract

The underlying mechanisms by which legume cultivars contribute to nitrous oxide (N_2O) generation are poorly understood. The aim of the present study was to explore the effects of two pea cultivars (Zero4 and Nitouche) intercropped with triticale, with or without wheat (*Triticum aestivum*) residues incorporation, on soil C and N dynamics, on bacterial community structure and their links with N_2O emissions. Monocrops and bare soil (no plant) treatments were used as an additional control in order to account for the level of mineralisation between treatments. Changes in total C and N contents and in some functionally-related soil pools (microbial biomass C and N, basal respiration, KCl-exchangeable ammonium and nitrate, potentially mineralizable N, DOC, ecophysiological indexes) were followed throughout a 97-day microcosm experiment carried out on a loamy arable soil. ARISA community fingerprinting of soil extracted DNA and GHG emissions were carried out at two key stages (pea flowering and harvest). The addition of residues to the soil resulted in only small changes to the total C and N pools the Nitouche monocrop, which was found to have the highest potentially mineralisable N (13.4 μg g^{-1} $28d^{-1}$) of the treatments with added residue. The different pea cultivar selectively affected N_2O emissions, with highest emissions associated with the cultivar Nitouche in the absence of residues. The two intercropping treatments of triticale/pea were significantly different either with residues or without, especially the triticale/Zero 4 which had the lowest values (356 g N_2O-N ha^{-1}). Similar patterns were also observed in below ground data. ARISA analysis showed that monocropped legumes and the Triticale-based treatment clearly grouped on separate clusters to the added residue treatment. We hypothesize that in pea-based intercrops variations in carbon supply from different cultivars may contribute to differences in N_2O emissions and thus influence the choice of suitable cultivars, to optimize nutrient cycling and sustainable crop management.

Keywords

bacterial community structure, C and N pools, N_2O emissions, pea-based intercropping, wheat residues

Introduction

Legume cropping offers opportunities to reduce GHG emissions from agriculture through their ability to substitute inputs of mineral fertilisers with biologically fixed N (Rochette *and* Janzen 2005). However, legumes differ widely in the their contribution to N_2O emissions and in some cases (particularly following residue incorporation) can still remain a significant source (Baggs *et al.*, 2000; Bouwman *et al.*, 2002). The cultivation of leguminous crops in agricultural systems can not only contribute to reducing the emission of nitrous oxide (N_2O) but also increases the release and the turnover of mineralisable N-containing compounds in soil (Rochette *and* Janzen 2005; Jensen *et al.*, 2010). Their ability to add external N to the plant-soil system is a distinct benefit on which crop production systems can rely on in order to maintain the soil N supply at a sustained productive level (Watson *et al.*, 2011). The amount of biologically fixed N supplied by legumes varies greatly from tens to several hundred kilograms per ha per year and is strongly affected by the type and environmental conditions (nitrate availability, temperature, soil wetness, and the availability of other nutrients).

Although symbiotic Rhizobium is believed to be able to produce N_2O in root nodules there is a conflicting evidence regarding the magnitude of this process. In their early work, O'Hara and Daniel (1985) suggested that rhizobial microorganisms are directly involved in the production of N_2O by reduction of NO_3 occurring within the root nodules. However, it is likely that Rhizobium species are not directly involved in the N_2O production process, and that the root microflora also plays an important role. Okubo *et al*. (2009) have shown that the rhizosphere community structure is significantly influenced by plant species and cultivar. It is also likely that this community structure is influenced by environmental conditions. It has been shown that different nodulation phenotypes contain different bacterial and fungal profiles in the stems and roots (Ikeda *et al.*, 2008). However, the extent to which these phenotypes are associated with different emissions is unclear. In the case of legumes, it has been suggested that N_2O emission is primarily associated with decomposition and turnover of root nodules (Inaba *et al.*, 2009), which implies that differences in the community structure and activity of root surface microorganisms may be responsible.

Understanding the contribution of legumes to N_2O emissions in the wider environment is highly dependent on developing an improved understanding of the underlying microbiology of the system (Philippot *et al.*, 2002). Many studies have been conducted involving legume based cropping systems especially placed in intercrops or the growing of two or more species together at one time, since, legume-based intercropping is able to provide several agro-ecological services: a more efficient use of soil resources for plant growth due to a reduced competition for soil N (Hauggaard-Nielsen *et al.*, 2003; Knudsen *et al.*, 2004; Hauggaard-Nielsen and Jensen, 2005), an increased water and nutrient use efficiency (Hauggaard-Nielsen *et al.*, 2009a), a greater yield stability and higher N concentration in cereal grain (Hauggaard-Nielsen *et al.*, 2006, 2009b), a better control of soil erosion (Inal *et al.*, 2007), and an enhanced weed suppression and pest control (Liebman and Dyck, 1993; Corre-Hellou *et al.*, 2011). Moreover, reduced N_2O emissions from soil (Pappa *et al.*, 2011) were also shown in leguminous intercrops. One more justification for intercropping (especially pea-based) is the increased mineral N made available in the soil for the following crop (Pappa *et al*., 2011; Scalise *et al.*, 2015). Finally, the legume cultivar has been shown to play an important role in the cumulative N_2O emissions of the agricultural systems, which also affects the product intensities (Pappa *et al*., 2011), which are all the emissions divided by all saleable outputs.

The aim of this study was to explore the mechanisms responsible for N_2O emissions from two legume species demonstrated by Pappa *et al*. (2011) by monitoring a number of soil chemical (pH; EC; C_{org}; Nt; NH_4^+-N; NO_3^--N; DOC), biochemical (MBC; R_{bas}; C_0, potentially mineralisable C; MBC/C_{org}; *q*M, mineralisation coefficient; *q*CO_2; *q*CO_2/C_{org} ratio; MBN; PMN, potentially mineralisable N) variables together with the bacterial community structure
by ARISA fingerprinting of soil extracted DNA, and GHGs emissions (N_2O, CH_4, CO_2) in an arable soil as by a microcosms approach.

The present study tested the following three hypotheses: a) legume-based cropping systems and wheat residue incorporation can stimulate soil C and N cycling through the enhancement of the below-ground nutrient flow, b) GHG emissions from legume-based intercropping can be altered by

902 soil addition of wheat residues and c) even when showing a similar yield potential, the cultivar of a
903 same leguminous species can selectively influence the soil processes including the bacterial
904 community structure conditioned by the legume intercrop.

905 **2. Materials and methods**

906 *2.1 Soil type and plant material*

907 The soil used in the microcosm experiment was a loam collected from the Ap horizon (0-30 cm)
908 of an agricultural field cultivated under continuous winter wheat and located at the Bush Estate,
909 Edinburgh, Scotland (55°52'17.46" N, 3°12'24.27" W). The main soil properties were: sand 42%,
910 silt 34%, clay 24%; bulk density 1.2 ± 0.1 kg dm^{-3}; pH_{H2O} 6.19 ± 0.04; total organic C (C_{org}) 34.27
911 ± 1.22 g kg^{-1}; total N (N_t) 2.52 ± 0.08 g kg^{-1}; C:N ratio 13.62 ± 0.20; NH_4^+ - N 3.75 ± 0.40 mg kg^{-1};
912 NO_3^- - N 7.64 ± 0.50 mg kg^{-1}; Olsen P 18.2 ± 0.4 mg kg^{-1}; extractable K 202.0 ± 0.3 mg kg^{-1};
913 electric conductivity measured in a soil:water (1:2, w/v) mixture ($EC_{1:2}$ at 25°C) 0.10 ± 0.01 dS m^{-1}.
914 Following the winter wheat (*Triticum aestivum*) harvest (September 2011), residual straw was
915 chopped to 2-4 mm and stored before being used for soil amendment. The soil for filling the
916 microcosms was collected before starting the experiment (3rd October 2011), coarse sieved at < 4.7-
917 mm particle size and brought to approximately 30% gravimetric water content. Seeds of two
918 cultivars of spring pea (*Pisum sativum* L. cv. Nitouche and *Pisum sativum* L. cv. Zero4) were
919 provided by PGRO (UK); seeds of triticale (*Triticum aestivum* L. x *Triticosecàle* Wittm.) were
920 provided by APSOVSEMENTI s.p.a. (Pavia, I).

921 *2.2 Experimental set-up*

922 The microcosm study was carried out at Scotland's Rural College (SRUC), in Edinburgh,
923 between October 2011 and February 2012. Microcosm units consisted of 2.12 L polyvinyl chloride
924 (PVC) pipes (25 cm height, 10.4 cm internal diameter) that had been closed at the base with an air-
925 tight seal using a sheet of Plexiglas®. A sampling point for the gas collection (a three-way tap) was
926 placed at 23 cm depth from the surface of the microcosm. Microcosms were filled either with soil

927 (no residue addition) (unamended) or with a soil plus chopped wheat straw (400:1, w/w) mixture
928 (corresponding to a 6.3 t ha^{-1} addition rate at a field scale) (wheat residue addition) (amended).
929 The amount of soil needed was calculated by taking into account the microcosm volume
930 (1867.92 cm^3), the soil bulk density and the gravimetric water content in order to reach a water-
931 filled pore space (WFPS) equal to 28-32% that provides optimum conditions for biological activity
932 in soil (FAO, 2001). WFPS was kept constant during the growing season by watering with a N-free
933 artificial rainwater (Palmqvist and Dahlman, 2006) in order to maintain suitable conditions for plant
934 growth and microbial processes without providing an external N addition.
935 Soon after filling (7th October 2011), each microcosm, four seeds were initially sown but only
936 two plants, of the same species or one of each intercrop components, were kept after successful seed
937 germination. For each level of soil amendment, the following six treatments compared different
938 combinations of leguminous intercrops and the respective sole crop: i) Nitouche: monocrop of pea
939 cv. Nitouche; ii) Zero4: monocrop of pea cv. Zero4; iii) Triticale: monocrop of Triticale; iv)
940 Nitouche-Triticale: intercrop pea cv. Nitouche-Triticale; v) Zero4-Triticale: intercrop pea cv. Zero4-
941 Triticale and vi) bare soil: unplanted microcosms were used as a control.
942 Since the scheduled samplings were destructive, the whole experiment was duplicated, giving a
943 total of 72 microcosms: (6 treatments) x (2 levels of amendment) x (2 samplings) x (3 replicates).
944 The microcosms were randomly arranged in a growth chamber and grown for a 97-day growing
945 period under controlled climatic conditions, as shown in Table 1.

946 *2.3Soil sampling and analysis*

947 Soil samples were collected at three sampling times: at the beginning (pre-sowing), at pea
948 flowering (62 days after sowing (DAS)) and at the pods filling pea stage (97 DAS), when the
949 microcosms were destructively sampled for soil and plant collection. Each microcosm provided one
950 rhizosphere sample (two samplings) and one bulk soil sample (three samplings). The rhizosphere
951 soil was taken from the plant roots after the bulk of the soil had been removed. The rhizosphere soil
952 was used for the molecular analysis and the bulk soil was used for the chemical and biochemical

characterization.

Soil chemical properties were determined according to standards methods recommended by the Soil Science Society of America (Sparks, 1996). Dissolved organic carbon (DOC) was extracted with water (1:2 w/v, soil:water) after shaking (170 rpm, 30 min) at room temperature. The soil slurries were then centrifuged (4300 rpm, 10°C, 10 min) and the recovered supernatant was filtered through a 0.45 µm Whatman GF/F membrane. DOC in the clean extract was finally measured using an automated elemental OC analyzer (Rosemount-Dohrmann DC-80) (Jones *et al.*, 2005) using a perchlorate oxidation followed by detection of CO_2 by NIR spectroscopy. Inorganic-N (NO_3^--N and NH_4^+-N) was extracted with 1 M KCl (1:5, w/v, soil:solution) after shaking (220 rpm, 60 min) at 24°C. After the extraction, the soil slurries were centrifuged (4300 rpm, 10 min) and the clean supernatants recovered and stored at -20°C before analysis. Inorganic N was determined using a continuous flow auto-analyser (SKALAR San^{++}, BV, NL).

Microbial biomass C (MBC) and N (MBN) were determined following a chloroform fumigation-extraction (CFE) procedure according to Vance *et al.* (1987) and Brookes *et al.* (1985). MBC was estimated using a conversion factor of K_{EC} = 0.45 (Joergensen, 1996) and MBN was estimated using a conversion factor of K_{EN} = 0.54 (Joergensen and Mueller, 1996). Soil basal respiration was estimated by measuring CO_2 emissions in sealed 1.5 L jars containing 20 g (dw equivalent) soil samples and incubated in the dark at 24 °C. Gas samples were collected in pre-evacuated 22 ml vials and analysed by gas chromatography (Sparling, 1981). The cumulative CO_2-C evolved after a 28-day incubation period (gas sampling was carried out after 1, 4, 7, 14, 21 and 28 days) was assumed as R_{bas}. The potentially mineralisable C (C_0) was estimated by fitting the 28-day cumulative data to the first-order exponential function $C_t=C_0\ (1-e^{-kt})$ (Riffaldi *et al.*, 1996). The best fitting of the equation to the values experimentally obtained and estimates of C_0 and k parameters for each curve of basal respiration were obtained by non-linear regression analysis using the Levenburg-Marquardt algorithm (Table Curve 2D v 5.01 software, SYSTAT software Inc.). Potentially mineralisable N (PMN), resulting from net mineralization of active soil organic N

occurring during the 28-day incubation period for R_{bas} determination, was estimated as the cumulative inorganic soil N after 28 days *minus* the inorganic soil N at 0 day (Drinkwater *et al*., 1996). The following soil eco-physiological indices were then calculated: the microbial quotient (MBC/C_{org}), the metabolic quotient (qCO_2), the mineralization coefficient ($qM=R_{bas}/C_{org}$) and the qCO_2/C_{org} ratio (Dilly *et al.,* 2001; Mocali *et al*., 2009).

DNA extraction from both rhizosphere and bulk soil were undertaken by ball milling samples to achieve physical lysis followed by a CTAB-buffer extraction method as described by Brierley *et al.* (2009). DNA extracts were purified from any humic acids by passing them through micro Bio-spin columns loaded with polyvinylpyrrolidone (PVP). DNA yield and quality were quantified by a spectrophotometer (ND-1000). Automated ribosomal intergenic spacer analysis (ARISA) was carried with an end-point PCR technique using the primer system 1406f (5'-TGYACACACCGCCCGT-3') and 23Sr (5'-GGGTTBCCCCATTCRG-3'). The PCR reaction mixture was prepared with GoTaq® Green Master Mix (Promega), 2 µl of template DNA (ca 20 ng), 0.5 µM of each primer, and sterile deionised water to a final volume of 25 µl. In the negative control, the tDNA was substituted with the same volume of nuclease-free water (Promega). PCR running conditions started with a single denaturation step of 94 °C for 3 min, to activate the HotStart enzyme, followed by 29 thermal cycles consisting of a denaturation step at 94 °C for 45 s, an annealing step at 55 °C for 1 min, and an elongation step at 72 °C for 2 min, followed by a final primer extension at 72 °C for 7 min and cooling to 4 °C. Capillary electophoresis with peaks ranging from 50-bp to 1,050-bp was carried out using an DNA 7500 assays on the Agilent 2100 Bioanalyzer (Analysis Software 2100, Agilent Technologies, Böblingen, D) according to manufacturer instructions. Electropherograms were imported into BioNumerics® 7.0 software package (AppliedMaths, Sint-Martens-Latem, B) as a 2D gel image for further analysis.

2.4 Greenhouse gas monitoring

Emissions of N_2O, carbon dioxide (CO_2) and methane (CH_4) from the microcosm units were

1005 measured following three gas sampling strategies: soil surface emissions, deep layer emissions (23
1006 cm) and respiration from roots. Surface gas monitoring started 12 days after sowing and was
1007 repeated (twice a week) across the entire experimental period by using the closed chamber
1008 technique (Smith *et al.,* 1995). During the gas emission measurements, the microcosms were
1009 covered by a 26-cm-tall chamber for 40-60 minutes before collecting 40 ml gas samples in a
1010 portable pre-evacuated 22-ml-glass vial (Scott *et al.,* 1999). For baseline corrections two air
1011 samples from the growing chamber atmosphere were collected at each sampling time. Gas sampling
1012 from deep soil layers started 38 days after sowing (14th November 2011) to allow time for the roots
1013 to grow throughout the microcosm and was repeated twice a week for three weeks. Gaseous
1014 emissions from legume roots collected after the microcosm destructive sampling (see below) were
1015 measured as described by Inaba *et al.* (2009). Shortly after the harvest, unwashed legume roots were
1016 placed into a 320 ml air-tight glass jars; 0 and 10 min after sealing, a 40-ml-gas sample was
1017 collected from the glass jar and immediately transferred in a pre-evacuated 22 ml glass vial. All gas
1018 samples were stored (maximum 1 day) in a controlled temperature room before any analysis.
1019 Amounts of N_2O, CO_2 and CH_4 of collected air samples were analyzed using an Agilent 6890 gas
1020 chromatograph equipped with a 1.8 m Propak-N column and an electron capture detector (for N_2O)
1021 and flame ionisation detector (for CH_4). Certified high purity gas standards of known concentration
1022 were used for calibration. The conversion of peak areas to daily gaseous emissions was carried out
1023 in accordance with standard procedures (de Kleine *and* Harvey, 2013). In addition, greenhouse gas
1024 emission intensities were expressed per unit of product (all emissions divided by all saleable
1025 outputs. Also the Global Warming Potential (GWP) of each gas was calculated using coefficients of
1026 1 for CO_2, 25 for CH_4 and 298 for N_2O.

1027 *2.5 Plant sampling and analysis*

1028 At pea flowering (62 DAS) and pod filling (97 DAS), the microcosms were destructively
1029 sampled, plants were gently removed from the microcosm soil and separated into shoot and root
1030 fractions. Shoot fresh weight was immediately recorded, whereas the root system was initially used

for measuring the N_2O emissions (legumes only). The above ground biomass results were used for the emission intensities calcilations.

2.6 Statistics

Soil variables were firstly checked for deviations from normality (Shapiro Wilk's test) and homogeneity of within-group variances (Levene's test). The block effect in the experimental design was not significant ($P > 0.05$) and the data were subjected to the following statistical analyses. A three-way analysis of variance (ANOVA) (treatment (T) x amendment (A) x time (Ti)), indicated in Figs. 1, 2 and 4 and in Table 2 as *F*-values and corresponding *P*-values, was performed in order to highlight the main effect of sampling time, crops, level of amendment and their interactions on measured soil variables. Significant effects due to treatment (T), amendment (A), and their interaction presented in Tab. 4 were estimated by a two-way ANOVA. Multiple pairwise comparison of means were assessed by Tukey's HSD (Honestly Significant Difference) test at $P < 0.05$ level of significance. Chemical and biochemical data were also analysed by principal component analysis (PCA) with no rotation with data from three different stages (pre-sowing, flowering and harvest) (Table 3 and 4). Statistical analyses were run using the Systat 11.0 software (SYSTAT Software Inc., Erkrath, D). Graphs were drawn by using the SigmaPlot 10.0 software (SYSTAT Software Inc.). Dendrograms of hierarchical classification of ARISA profiles were generated by cluster analysis using the unweighed pair-group method with arithmetic averages (UPGMA) based on Dice similarity coefficient as suggested by Rademaker *et al.* (1999).

3. Results

3.1 Soil C pools

Soil carbon pools showed variable responses to the addition of plant residues and the presence of different crop cultivars during the experiment. The addition of wheat residues in the microcosm soils caused some significant reductions in the amount of the total organic carbon (C_{org}) (Table 2), although residue incorporation affected the C_{org} differently in treatments over time. In particular, in

1056 unamended soils, C_{org} values remained close to the initial values; whereas following wheat residue
1057 addition, a contrasting affect was observed in C_{org} content between monocropped treatments were
1058 found to have the highest C_{org} concentrations. In bare soil C_{org} slightly declined, whereas it
1059 remained practically unaffected in amended ones.

1060 Dissolved organic carbon (DOC) varied in response to residue addition levels and sampling
1061 stages (Fig. 1). The presence of the intercrops increased the concentrations of DOC at harvest.
1062 Without residues addition, no significant difference was observed between treatments at any
1063 sampling stage; whereas following wheat residue addition the Zero4 treatment showed a significant
1064 increase ($P < 0.001$) from pre-sowing (36.9 μg g^{-1}) to harvest (64.1 μg g^{-1}). On average, DOC
1065 increased over time from an initial value of 33.2 (or 37.3) to 48.2 (or 50.5) μg g^{-1} in unamended (or
1066 amended) microcosms soil, including the bare soil which showed an increasing trend over time.

1067 In unamended microcosms, mean soil basal respiration, R_{bas}, values were higher than pre-
1068 sowing at both flowering and harvest stage (respectively 778.9 and harvest 807.4 μg CO_2-C g^{-1} 28 d^{-1})
1069 and there was no significant effect due to the crop treatment (Fig. 1). However, residue
1070 amendment strongly influenced ($P < 0.05$) the CO_2 emission of treatments at the harvest stage,
1071 which ranged between 553.5 (bare soil) and 1042.6 μg CO_2-C g^{-1} 28 d^{-1} (Nitouche monocropping):
1072 the Nitouche solo crop showed higher basal respiration than those at beginning of the experiment
1073 (from 721.7 to 1042.6 μg CO_2-C g^{-1} 28 d^{-1}), whereas in the bare soil R_{bas} decreased by
1074 approximately 20% (from 664.6 to 553.5 μg CO_2-C g^{-1} 28 d^{-1}). Estimates of the potentially
1075 mineralisable carbon (C_0) followed the same general trend as those of R_{bas}, even though some of the
1076 experimental factors lost their significance (Fig. 1). It is noteworthy that C_0 displayed a time-
1077 dependent fluctuation with particularly high C mineralization from Triticale (differing from R_{bas})
1078 and Nitouche monocrops.

1079 Microbial biomass carbon (MBC) was strongly affected by treatments with statistically
1080 significant responses to all the experimental factors (Fig. 1). In general the MBC increased during
1081 the cropping season, in spite of residue amendment: from an initial 79.0 (or 75.5) to final 191.4 (or

1082 243.6) μg C g^{-1} in unamended (or amended) soil microcosms. In soils with no wheat residue
1083 addition, MBC showed a large increase in the presence of legume-based treatments either in
1084 monocropped - from mean initial 79.1 to final 209.3 μg C g^{-1} (approximately +265%) - or
1085 intercropped legumes - from initial 75.5 to final 233.8 μg C g^{-1} (approximately +310%). An
1086 opposite affect was observed in residue amended soils: the MBC increase was generally lower
1087 under intercropping (+290%) than in monocropping (+390%) as compared with the starting value of
1088 75.7 μg C g^{-1}.

1089 *3.2 Soil N pools*

1090 Time and time x amendment were the only factors that significantly affected the variability of
1091 total nitrogen content (N_t) in microcosms soils (Table 2). In fact, N_t content decreased across the 97-
1092 day experimental period with differing trends, but reaching similar values at the harvest stage (2.09
1093 and 2.01 g kg^{-1} for unamended and amended, respectively) (data not shown). Across the
1094 experimental period, the extractable NH_4^+-N did not differ significantly in any of the treatments
1095 (Fig. 2); however, the amount of soil nitrate showed marked time-dependent fluctuations and was
1096 significantly different among treatments ($P < 0.001$). Crop growth markedly affected the dynamics
1097 of soil nitrate-N, which became greatly depleted at the flowering stage in all planted microcosms.
1098 An increased release of nitrate was observed at the latest stage, also mirrored by a decline in the
1099 ammonium-N content, yet regulated by the decaying wheat residues (Fig. 2).

1100 The potentially mineralisable nitrogen (PMN) was affected by all the experimental factors and
1101 their interactions (Fig. 2). In general, PMN demonstrated a clear decrease from pre-sowing onward.
1102 At the flowering stage, PMN in the bare soil treatment was significantly higher ($P < 0.01$) than the
1103 treatments with no residue addition. It was noteworthy that, at the harvest stage, PMN was
1104 significantly affected by residue amendment, even though at a different level ($P < 0.05$ and $P <$
1105 0.001, respectively). Specifically, Nitouche monocropping increased the PMN by three times from
1106 the flowering stage reaching the highest value of 13.4 μg g^{-1} $28d^{-1}$ in microcosms packed without
1107 addition of wheat residues. All the remaining cropping treatments showed a small non-significant

1108 increase, but the bare soil retained similar values (10.81 μg g^{-1} 28 d^{-1}). Further, in amended soils,
1109 there was a significant increase in the Triticale - Zero4 intercropping from the flowering (5.8 μg g^{-1}
1110 28 d^{-1}) to the harvest stage (12.3 μg g^{-1} 28 d^{-1}).

1111 All experimental factors and their interactions statistically influenced the microbial biomass N
1112 ($P < 0.001$). In unamended microcosms, MBN moderately (intercrops) or strongly (monocrops)
1113 increased over time, with the exception of the bare soil treatment where it decreased from the
1114 beginning of the experimental period (14.6 μg N g^{-1}) by approx. 40% (from 14.6 to 8.8 μg N g^{-1}). In
1115 contrast, in residue amended soils, the unplanted soil showed MBN values statistically comparable
1116 to other cropping treatments: as a whole MBN increased by approx. 70%, from initial 17.0 to final
1117 28.8 μg N g^{-1} (Fig. 2).

1118 *3.3 Soil ecophysiological indices and C-to-N ratios*

1119 The mineralization coefficient (*q*M) was statistically influenced by the amendment level ($P <$
1120 0.001), time ($P < 0.001$) and their interactions (Fig. 3). In unamended soils, the mineralization
1121 coefficient values showed a slight increase, on average from 16.79 μg CO_2–C mg^{-1} C_{org} (pre-sowing
1122 stage) to 24.35 μg CO_2–C mg^{-1} C_{org} (harvest sampling). In microcosms added with wheat residues,
1123 it showed an opposite trend for Zero4, Triticale and bare soil, which showed the major decline
1124 (from 25.14 μg CO_2–C mg^{-1} C_{org} to 18.80 μg CO_2–C mg^{-1} C_{org}).

1125 The metabolic quotient (qCO_2) was significantly ($P < 0.001$) affected only by time, level of soil
1126 amendment and their interaction (Fig. 3). Microcosms at both level of amendment showed a
1127 decrease in the values of the qCO_2 towards the end of the experiment, which was stronger in the
1128 amended soil due to the higher average values it showed in the pre-sowing stage (1.11 and 2.57 μg
1129 CO_2–C μg^{-1} MBC d^{-1} respectively for unamended and amended). The largest decrease was
1130 registered in the Nitouche pure culture (from 2.69 to 0.19 μg CO_2–C μg^{-1} MBC d^{-1}). The qCO_2/C_{org}
1131 ratio was also statistically influenced by time ($P < 0.001$), level of soil amendment ($P < 0.001$) and
1132 their interactions (Fig. 3). However, in amended microcosms, the qCO_2/C_{org} ratio clearly decreased
1133 in all treatments from pre-sowing to harvest stage.

1134 The microbial quotient (MBC/C_{org}) was strongly ($P < 0.001$) affected by all the experimental
1135 factors (Fig. 3). MBC/C_{org} varied consistently during the experimental period and showed a marked
1136 increase at the harvest stage in all treatments at both amendment levels. The bare soil treatment
1137 always showed the lowest value within each sampling time, reaching its minimum at the flowering
1138 stage in residue amended microcosms (2.09 µg MBC mg^{-1} C_{org}).

1139 *3.4 Soil pH and electrical conductivity*

1140 The three-way ANOVA revealed that wheat residue addition was the main factor affecting the
1141 variability of pH data ($P < 0.001$), which were generally higher in the amended soil (Table 2). There
1142 were also a time-dependent fluctuations ($P < 0.01$) together with significant effects of the
1143 amendment x time, and amendment x time x treatment interactions ($P < 0.001$). However, the pH
1144 varied between a narrow range comprised between 6.12 (unamended bare soil at flowering) and
1145 6.37 (amended triticale at flowering), and significant differences among treatments were only
1146 noticed at the flowering and the harvest stages in the unamended soil with the Nitouche monocrop
1147 and bare soil having, respectively, the highest (6.39) and the lowest value (6.10).

1148 The electrical conductivity ($EC_{1:2}$) varied between 0.10 and 0.18 $dS \cdot m^{-1}$ and was significantly
1149 affected by most of the experimental factors and their interactions (Table 2). It was noticeable that
1150 the triticale-based treatments showed higher $EC_{1:2}$ values than the leguminous sole treatments at
1151 both flowering and harvest stages: this finding was only observed in unamended, but not in the
1152 amended microcosms, and this was especially true for all crop-based treatments where EC remained
1153 almost constant over time. In the bare soil, the lowest EC was found in unamended treatments
1154 (~0.10 dS m^{-1}); whereas following wheat residues addition it increased considerably at flowering
1155 and harvest stage, respectively to 0.18 and 0.15 dS m^{-1}.

1156 *3.5 Multivariate analysis*

1157 According to the eigenvalue > 1.0 criterion only five principal components could be selected. The
1158 first two principal components PC1 (eigenvalue 5.37) and PC2 (eigenvalue 2.79) explained a large

1159 portion (33.55 and 17.44%, respectively) of the total variance. The following three components PC3
1160 (eigenvalue 2.17), PC4 (eigenvalue 1.35) and PC5 (eigenvalue 1.06) accounted for 13.54, 8.43 and
1161 6.63% of total variance, respectively. Since the first two components taken together explained as
1162 much as 50.98% of the total variance, we focused on them (Table 3). Firstly, it is worth noting that
1163 PC1 was primarily weighed by either C-related functional variables (DOC, *q*M, R_{bas}, MBC and
1164 MBC/ C_{org}) or N-related variables (PMN and N_t). It was also found that PC2 was primarily affected
1165 by one of the most dynamic N pools in soil: NH_4^+-N, which was also directly related to qCO_2 and
1166 qCO_2/C_{org}. On the other hand MBN was the only variable affecting PC3. Moreover, PC4 was
1167 weighed by C_{org} and pH. Whereas, in PC5 PMC was the only soil variable showing a loading factor
1168 close to the reference threshold value (0.60). In the ordination biplot of Factor 1 vs Factor 2, soil
1169 samples from the differing treatments appeared in most cases well separated at least in three main
1170 groups along the PC axis 1 (functional C variables and N-related properties): triticale
1171 monocropping, Nitouche - Triticale intercropping and, surprisingly, a rather broad group including
1172 all the other crop treatments plus the bare soil. On the other hand, the two leguminous monocrops
1173 were clearly separated along the PC axis 2 (Fig. 4A).
1174 The two first principal components PC1 (eigenvalue 5.21) and PC2 (eigenvalue 2.92) expressed a
1175 somewhat large portion (32.59 and 18.22%, respectively) of the total variance. The following three
1176 components PC3 (eigenvalue 2.04), PC4 (eigenvalue 1.46) and PC5 (eigenvalue 1.22) accounted
1177 for 12.77, 9.14 and 7.60% of total variance, respectively. Once again, we focused on the first two
1178 PCs as they explained as much as half of the variance (50.81%) (Table 4). PC1 was primarily
1179 weighed by either C-related functional variables (MBC, MBC/C_{org}, qCO_2/C_{org}, qCO_2 and DOC) or
1180 N-related variables (MBN, PMN and N_t). PC2 was primarily affected by some C-related functional
1181 variables (R_{bas}, *q*M and C_0) and NO_3^--N. C_{org} was the only variable affecting PC3. EC was the only
1182 variable affecting PC4.. In the ordination biplot of Factor 1 vs Factor 2, soil samples from the
1183 amended microcosms were rather scattered onto the plot: the two intercropping combinations were
1184 closely associated, whereas the two leguminous monocrops were not. The bare soil was well

separated from the other treatments. Noticeably, among the chemical and biochemical soil variables, NO_3^--N and C (C_0) exerted a primary role in separating the treatments along the PC2 axis (Fig. 4B).

3.6 ARISA analysis

The molecular structure of the bacterial communities profiles were characterized by the number and length distribution of major bands which, in spite of treatments and residue levels, were observed in a fragment size range from 200 to 1000 bp, and showed a clear diversity between levels of residue. In particular, regardless of the growth stage, residues addiction in soils appeared to enhance the difference in groups allowing the monocropped legumes and Triticale-based treatment to clearly group on separate clusters (~78%; Fig. 5). On the contrary, in the no-residue soils, the treatment-dependent communities did not clearly align on the endemic axis, not allowing the clusters to present a clear pattern. The only clear difference was between bare soil and other treatments, which showed a level of similarity of approximately 73%.

3.7 Greenhouse gases (GHGs) emissions

Nitrous oxide emissions from the amended treatments were lower in comparison to the unamended soils ($P < 0.001$). In the amended treatments, the emissions started to pick up after 60 days of the start of the experiment with the unplanted treatment having the highest emissions (81.25 g N_2O-N ha^{-1} day^{-1}). In the unamended treatments, the emissions were higher ($P < 0.05$) in the Triticale monocrop and Triticale/Nitouche treatments including also the no plant treatment from 30 days after the seeding.

The cumulative values of N_2O were higher in the unamended treatments at 82 days. The bare soil treatment had the highest emissions in both treatments (4319 and 1430 g N_2O-N ha^{-1} in unamended and amended, respectively). In the microcosms with crop, the Triticale/Nitouche treatment had the highest (3677 g N_2O-N ha^{-1}) and the Triticale/Zero 4 the lowest (356 g N_2O-N ha^{-1}) emissions in unamended soils ($P < 0.001$). In the amended treatments, the cumulative emissions were generally

very low, and even showed consumption of N_2O (negative values) with similar patterns in the unamended soils (Triticale/Nitouche: 243 g N_2O-N ha^{-1} and Triticale/Zero4: -550 g N_2O-N ha^{-1}) (Table 5). Below ground N_2O emissions showed a similar pattern between amendment levels during the experimental period. However, the concentration of N_2O was ten times greater from the no residue treatment in comparison with the residue ($P < 0.001$). The bare soil treatment had the highest average values (19.70 ppm and 1.95 ppm for the no residue and residue, respectively) followed by the Triticale/Nitouche treatment (2.56 and 1.30 ppm for the unamended and amended, respectively) (Table 5).

Cumulative CO_2 emissions were highest in the Zero 4 treatment (2511 kg CO_2-C ha^{-1}) in unamended soils and the Nitouche (2790 kg CO_2-C ha^{-1}) under residue addition (Table 5). The bare soil treatment had the highest average belowground concentration of CO_2 in both residue treatments during the experimental period ($P < 0.001$) (Table 5). Methane emissions were low during the experimental period for both level of amendment without (Table 5).

Emission intensities presented in this paper include the cumulative N_2O measurements (84 out of 97 days) for the total biomass produced within this time providing an index of the effectiveness of mitigation. In the residue treatment, the Triticale/Zero4 had the lowest emission intensities of all the treatments (-393 g N_2O t $biomass^{-1}$). N_2O intensities were not significant different for the no residue treatment (Table 6).

4. Discussion

The results obtained from this study provide new insights into the interrelated effects of leguminous crops on the chemical and biochemical properties of soil and highlights the important differences in C and N cycling associated with pea-based intercropping and wheat residue incorporation.

4.1 Soil chemical properties

Even in simplified ecosystems such as microcosms, soil organic carbon can be considered one of

1235 the most important indicators of soil quality because of its important role in the maintenance of soil
1236 structure, microorganisms and nutrient cycling (Aalders *et al*., 2009).

1237 Soil incorporation of wheat residues slightly reduced the total organic carbon (C_{org}), which
1238 appeared noticeable in the ANOVA analysis but resulted negligible impacts in the principal
1239 component analysis either with or without residue addition. Indeed, it could be anticipated that total
1240 soil organic matter, would not respond rapidly to environmental changes, unless major amendments
1241 are made (Powlson *et al.,* 1987). However, mixing occurring during the establishment of the
1242 experimental units was expected to alter soil C dynamic and enhance rates of soil organic matter
1243 degradation, thus leading to a so-called tillage effect (Linsler *et al.,* 2013; Tortorella and Gelsomino,
1244 2011). The observation that soils receiving residue inputs were associated with lower organic C and
1245 N pools would indicate that the addition of residues had stimulated the microbial populations and
1246 increased the decomposition of preexisiting organic matter through a priming effect (Kuzyakov
1247 2002). This increased degradation activity not only influenced the carbon but also the nitrogen
1248 cycling, which is functionally interconnected in soil, and thus resulted in a more striking variation
1249 in N_t than in the C_{org}.

1250 Even if major changes in total organic carbon content may be difficult to detect over a short-term
1251 experiment (Haynes, 1999), the responses of more labile fractions of soil organic carbon, namely
1252 dissolved organic carbon (DOC), are much more sensitive to soil management than total soil
1253 organic matter (Silveira, 2005). This fraction markedly influences soil chemical, biological and
1254 physical properties, as a primary source of mineralizable C, N, P, and S (Haynes, 2000) and it has
1255 been proposed as an indicator of the size of the available C pool to soil microorganisms (Boyer and
1256 Groffman, 1996).

1257 Through their exudates, plant root systems represents a major source of C flow entering the soil
1258 and stimulating the microbial process of immobilisation/release of soluble organic compounds
1259 forming the DOC pool in soil (Paterson, 2003; Paterson *et al.,* 2007). In fact, the quality and
1260 amount of rhizodeposition released from the legumes root systems could explain the high

1261 significance showed by the crop factor on the variability of this parameter in this study (Fustec *et*
1262 *al*., 2010).

1263 The addition of plant residues and fresh organic compounds through rhizodepositions most often
1264 results in a net N immobilisation phase followed by a net re-mineralisation phase. In our study,
1265 lower amounts of inorganic N were observed in the treatment with wheat residue addition than in
1266 the corresponding unamended treatment. Wheat residue incorporation seems to have enhanced net
1267 N immobilization, although N mineralization was promoted in presence of the legume treatment at
1268 the end of the incubation period.

1269 The significant difference between amendment levels and crop presence shown suggests a
1270 different effect of faunal activity on residues. This could be due to increased available N in soil,
1271 which is consequently is not limiting for soil microorganisms responsible for degrading the
1272 residues. However, Knapp *et al*. (1983) reported conflicting evidence where some studies found
1273 mixed results from the effect of N availability on residue decomposition.

1274 Soil pH was fairly resilient to changes during the microcosm experiment (as clearly shown by
1275 PCA analysis) and this was actually not unexpected since it is not a highly variable parameter, and
1276 is often resilient also to short term perturbations (Table 3 and 4).

1277 *4.2 Soil biochemical properties*

1278 This study confirms, as previously suggested (Ndiaye *et al.*, 2000), that biological and
1279 biochemical parameters are more sensitive and can provide earlier measurements of changes
1280 produced by different soil and crop management than physical and chemical indicators. Most
1281 authors have studied the quantity and the activity of soil microbial biomass as indicator of changes
1282 driven by the addition of organic residue or cropping systems (Kaiser and Heinemeyer, 1993;
1283 Ndiaye *et al.*, 2000).

1284 Microbial biomass, is known to be one of the main drivers of nutrient cycling in soils, with
1285 microbial activity releasing essential nutrients to plants and microbial biomass is functionally and
1286 closely linked to the turnover of soil organic carbon (Jenkinson and Ladd, 1981). It is therefore of

1287 significance that the soil microbial biomass showed a greater increase, in all legume based
1288 treatments in the unamended soil. This increase, observed at the last sampling, could have been due
1289 to higher growth of microbial biomass, induced by the legume crop (Dinesh *et al*., 2004). The
1290 statistically significant difference shown in the microbial biomass dynamics in response to the
1291 presence/absence of residues can depend on the decomposition rate of plant material and on the
1292 microbial immobilisation processes. In fact, the N assimilation requirements are determined by this
1293 carbon flow (Mary *et al*., 1996). It is often assumed that N coming from the residue and from
1294 recycled biomass is mineralised before being assimilated by the newly-formed biomass. However, it
1295 has been shown that the soil microflora can directly assimilate significant amounts of organic N
1296 compounds coming from plant residues or from decaying biomass.

1297 Furthermore, the introduction of the residue amendment increased soil basal respiration as
1298 measured by cumulative CO_2 emissions. Although R_{bas} was not responsive to the individual
1299 treatments, it was markedly influenced by the interactions they determined with the amendment.
1300 This finding can suggest that in this soil the metabolic activity was primarily influenced by
1301 compositional changes in soil organic matter due an enhanced residue decomposition of the organic
1302 compounds released from plants roots.

1303 *4.3 Analysis of soil microbial community structures*

1304 The results obtained in this study confirm that the addition to the soil of crop residues can
1305 strongly modify the genetic structure of the community by stimulating particular populations;
1306 especially as the soil system is often substrate-limited as regards microbial growth (Nicolardot *et*
1307 *al*., 2007). In fact, the molecular analysis revealed that the genetic structures of the bacterial
1308 population itself were significantly changed in response to the presence of legume sole crops or
1309 triticale, either in association with the legume or in monocropping, as a function of the
1310 presence/absence of wheat residue in the soils.

1311 *4.4 Greenhouse Gas emissions*

Our study demonstrated that there were lower N_2O emissions from legumes, which is consistent with our understanding that there are low levels of N_2O emission associated with the fixation process (Rochette *and* Janzen 2005). The results are also consistent with those of Pappa *et al* (2011) showing higher emissions from the pea cultivar Nitouche both as a monocrop and when grown as an intercrop. The Nitouche monocrop had up to six time higher emissions (434 g ha^{-1}) than the monocrop Zero 4 (71 g ha^{-1}) in the amended treatment and twice in the unamended (749 and 374 g ha^{-1} for Nitouche and Zero 4, respectively). However, there was no significant difference between the intercropping treatments. Intriguingly these higher emissions were observed in the absence of wheat residue additions, and did not appear to be associated with elevated concentrations of DOC. The denitrification processes driven by the availability of oxidisable carbon, which is used as a terminal electron acceptor in the respiratory process. Therefore, the absence of higher levels of DOC in the legumes was elevated emissions of N_2O raises the possibility that the carbon was being supplied by the legume itself. Support for this hypothesis would be provided by higher soil respiration rates measured from Nitouche, even in the absence of acid plant residues and as indicated in differences in microbial activity shown by the ARISA analysis. There is also a growing body of evidence indicating that differences in rhizodeposition associated with different crop cultivars may drive differences in N2O emissions (Gogoi & Baruah 2012; Sey *et al.* 2010)

Plant species and combinations of species offer significant opportunities to modify soil derived N_2O emissions. If differential rates of rhizodeposition are able to alter denitrification rates, then selecting specific legume cultivars with low rates of deposition in combination with cereals may therefore provide a novel opportunity for the mitigation of N_2O emissions. It is possible that the mechanisms underlying these differences would be associated either with an improved capacity of certain legume cultivars to compete more efficiently for soil N. Alternatively there may be an interaction between the legume and soil microbial community that reduces N_2O emission (possibly by promoting increased rates of N immobilization). The choice of legume cultivar and species is therefore a key factor influencing the amount of N loss. A previous study (Pappa *et al*., 2011) has

shown that the cultivar Zero 4 has significant lower N loss by N_2O emissions and leaching and could therefore contribute to the development of agricultural systems with environmental benefits. Therefore having a better understanding of the varietal differences in selecting intercrops mixtures has a high potential to increase yields and contribute towards the developments of agricultural systems with environmental benefits.

5. Conclusions

Legumes are generally associated with lower emissions of N_2O than cereal crops. However, there is significant variability in emissions between different legume cultivars. In this study the higher emissions associated with Nitouche were generated in the absence of wheat residues, raising the possibility that this variation in emissions is driven by variations in carbon supplied from the legume root. The intercrop affect on microbial activity is also cultivar specific. This is indicated by differences in N_2O emissions observed from two pea cultivars when grown as intercrops, although differences in N_2O emission were not linked to differences in yield. The mechanism underlying these differences appears to be driven by the differences resulting from microbial activity, which in turn are likely to be linked to soil-plant carbon dynamics.

Our research therefore highlights the importance of the cultivar choice in the sustainable agricultural systems. The addition of the residues affects the soil C pools and the N_2O emissions and shows clear differences between the two pea cultivars but also the intercropping combinations. The root development of pea monocrops was influenced by the residue addition but also the presence of cereal highlighting the complexity of such systems. The scale of these effects is highly sensitive to management and soil type. The growing need for environmental tests of the legume cultivars to understand further the mechanisms of the GHGs emissions is in high priority. Understanding the development of legume cultivar and the interactions taking place within legume/cereal intercrop has the potential to be a very useful management tool in the development of more sustainable agricultural systems and in mitigation of GHG from agriculture.

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References

Aalders, I., Hough, R.L., Towers, W., Black, H.I.J., Ball, B.C., Griffiths, B.S., Hopkins, D.W., Lilly, A., McKenzie, B.M., Rees, R.M., 2009. Considerations for Scottish soil monitoring in the European context. Eur. J. Soil Sci. 60, 833-843.

Baggs, E.M., Watson, C.A., Rees, R.M., 2000. The fate of nitrogen from incorporated cover crop and green manure residues. Nutr. Cycl. Agroecosys. 56, 153-163.

Boyer, J.N., Groffman, P.M., 1996. Bioavailability of water extractable organic carbon fractions in forest and agricultural soil profiles. Soil Biol. Biochem. 28, 783-790.

Bouwman, A.F., Boumans, L.J.M., Batjes, N.H., 2002. Emissions of N_2O and NO from fertilized fields: Summary of available measurement data. Global Biogeochem. Cycles 16(4), 1058.

Brierley, J.L., Stewart, J.A., Lees, A.K., 2009. Quantifying potato pathogen DNA in soil. Appl. Soil Ecol. 41, 234-238.

Brookes, P.C., Landman, A., Pruden, G., Jenkinson, D.S., 1985. Chloroform fumigation and the release of soil nitrogen: a rapid direct extraction method to measure microbial biomass nitrogen in soil. Soil Biol. Biochem. 17, 837-842.

Corre-Hellou, G., Dibet, A., Hauggaard-Nielsen, H., Crozat., Y., Gooding, M., Ambus, P., Dahlmann, C., von Fragstein, P., Pristeri, A., Monti, M., Jensen, E.S., 2011. The competitive ability of pea-barley intercrops against weeds and the interactions with crop productivity and soil N availability. Field Crop Res. 122, 264-272.

de Kleine, C., Harvey, M., 2013. Nitrous oxide chamber guidelines; Global Research Alliance on Agricultural Greenhouse Gases. Ministry of Primary Industries, 25 The Terrace, PO Box 2526, Wellington, New Zealand.

Dilly, O., Winter, K., Lang, A., Munch, J.C., 2001. Energetic eco-physiology of the soil microbiota in two landscapes of southern and northern Germany. J. Plant Nutr. Soil Sci. 164, 407-413.

Dinesh, R., Suryanarayana, M.A., Ghosha, S., Sheeja, T.E., 2004. Long-term influence of leguminous cover crops on the biochemical properties of a sandy clay loam Fluventic

1401 Sulfaquent in a humid tropical region of India. Soil Till. Res. 77, 69-77.
1402 Drinkwater, L.E., Cambardella, C.A., Reeder, J.D., Rice, C.W., 1996. Potentially mineralisable
1403 nitrogen as an indicator of biologically active soil nitrogen. In: Doran J.W., Jones A.J. (Eds.),
1404 Methods for Assessing Soil Quality. Soil Science Society of America, Special Publication 49,
1405 Madison, WI. 217-229.
1406 FAO, 2001. Factors regulating nitrous oxide and nitric oxide emission. Available at
1407 http://www.fao.org/DOCREP/004/Y2780E/y2780e02.htm
1408 Fustec, J., Lesuffleur, F., Mahieu, S., Cliquet, J.B., 2010. Nitrogen rhizodeposition of legumes. A
1409 review. Agron. Sustain. Dev. 30, 57-66.
1410 Gogoi,B.& Baruah,K.K. 2012. Nitrous oxide emissions from fields with different wheat and rice
1411 varieties. Pedosphere, 22, 112-121.
1412 Haynes, R.J., 1999. Labile organic matter fractions and aggregate stability under short-term grass-
1413 based leys. Soil Biol. Biochem. 31, 1821-1830.
1414 Haynes, R.J., 2000. Labile organic matter as an indicator of organic matter quality in arable and
1415 pastoral soils in New Zealand. Soil Biol. Biochem. 32, 211-219.
1416 Hauggaard-Nielsen, H., Ambus, P., Jensen, E.S., 2003. The comparison of nitrogen use and leaching
1417 in sole cropped versus intercropped pea and barley. Nutri. Cycl. Agroecosys. 65, 289-300.
1418 Hauggaard-Nielsen, H., Jensen, E.S., 2005. Facilitative root interactions in intercrops. Plant Soil
1419 274, 237-250.
1420 Hauggaard-Nielsen, H., Andersen, M.K., Jørnsgaard, B., Jensen, E.S., 2006. Density and relative
1421 frequency effects on competitive interactions and resource use in pea-barley intercrops. Field
1422 Crop Res. 95, 256-267.
1423 Hauggaard-Nielsen, H., Mundus, S., Jensen, E.S., 2009a. Nitrogen dynamics following grain
1424 legumes and subsequent catch crops and the effect on succeeding cereal crops. Nutri. Cycl.
1425 Agroecosys. 84, 281-291
1426 Hauggaard-Nielsen, H., Gooding, M., Ambus, P., Corre-Hellou, G., Crozat, Y., Dahlmann, C.,

1427 Dibet, A., Von Fragstein, P., Pristeri, A., Monti, M., Jensen, E.S., 2009b. Pea-barley
1428 intercropping for efficient symbiotic N2-fixation, soil N acquisition and use of other nutrients in
1429 European organic cropping systems. Field Crops Res. 113, 64-71.
1430 Ikeda, S., Rallos, L.E.R., Ohkubo, T., Eda, S., Inaba, S., Mitsui, H., 2008. Microbial community
1431 analysis of field-grown soybeans with different nodulation phenotypes. Appl. Environ.
1432 Microbiol. 74, 5704–5709.
1433 Inaba, S., Tanabe, K., Eda, S., Ikeda, S., Higashitani, A., Mitsui, H., Minamisawa, K., 2009. Nitrous
1434 oxide emission and microbial community in the rhizosphere of nodulated soybeans during the
1435 late growth period. Microbes Environ. 24 (1), 64-67.
1436 Inal, A., Gunes, A., Zhang, F., Cacmak, I., 2007. Peanut/maize intercropping induced changes in
1437 rhizosphere and nutrient concentrations in shoots. Plant Physiol. Biochem. 45, 350-356.
1438 Jenkinson, D.S., Ladd, J.N., 1981. Microbial biomass in soil: Measurement and turnover. In: Soil
1439 Biochemistry vol 5 (Paul E.A., Ladd J.N., eds). Marcel Dekker, New York. pp. 415-471.
1440 Jensen, E.S., Peoples, M.B., Hauggaard-Nielsen, H., 2010. Faba bean in cropping systems. Field
1441 Crops Res. 115, 203-216.
1442 Joergensen, R.G., 1996. The fumigation-extraction method to estimate soil microbial biomass:
1443 calibration of the K_{EC} value. Soil Biol. Biochem. 28, 25-31.
1444 Joergensen, R.G., Mueller, T., 1996. The fumigation-extraction method to estimate soil microbial
1445 biomass: calibration of the K_{EN} value. Soil Biol. Biochem. 28, 33-37.
1446 Jones, D.L., Healey, J.R., Willett, V.B., Farrar, J.F., Hodge, A., 2005. Dissolved organic nitrogen
1447 uptake by plants. An important N uptake pathway? Soil Biol. Biochem. 37(3), 413-423.
1448 Kaiser, E.A., Heinemeyer, O., 1993. Seasonal variations of soil microbial biomass carbon within the
1449 plow layer. Soil Biol. Biochem. 25, 1649-1655.
1450 Knapp, E.B., Elliott, L.F., Campbell, G.S., 1983. Carbon, nitrogen and biomass interrelationships
1451 during the decomposition of wheat straw: a mechanistic simulation model. Soil Biol. Biochem.
1452 15, 455-461.

1453 Knudsen, M.T., Hauggaard-Nielsen, H., Jørnsgaard, B., Jensen, E.S., 2004. Comparison of
1454 interspecific competition and N use in pea-barley, faba bean-barley and lupin-barley intercrops
1455 grown at two temperate locations. J. Agric. Sci. 142, 617-627.
1456 Kuzyakov,Y. 2002. Review: Factors affecting rhizosphere priming effects. Journal of Plant
1457 Nutrition and Soil Science-Zeitschrift fur Pflanzenernahrung und Bodenkunde, 165, 382-396.
1458 Liebman, M., Dyck, E., 1993. Crop rotation and intercropping strategies for weed management.
1459 Ecol. Applic. 3, 92-122.
1460 Linsler, D., Geisseler, D., Loges, R., Taube,F., Ludwig, B., 2013. Temporal dynamics of soil organic
1461 matter composition and aggregate distribution in permanent grassland after a single tillage event
1462 in a temperate climate. Soil Till. Res. 126, 90-99.
1463 Mary, B., Recous, S., Darwis, D., Robin, D., 1996. Interactions between decomposition of plant
1464 residues and nitrogen cycling in soil. Plant Soil. 181, 71-82.
1465 Mocali, S., Dentice, A., Marcucci, A., Benedetti, A., 2009. The impact of post-harvest treatments of
1466 transgenic eggplant residues on soil quality and microbial diversity. Agrochimica. 53(5), 296-
1467 307.
1468 Ndiaye, E.L., Sandeno, J.M., Mcgrath, D., Dick, R.P., 2000. Integrative biological indicators for
1469 detecting change in soil quality. Am. J. Altern. Agric. 15, 26-36.
1470 Nicolardot, B., Bouziri, L., Bastian, F., Ranjard, L., 2007. Influence of location and quality of plant
1471 residues on residue decomposition and genetic structure of soil microbial communities. Soil
1472 Biol. Biochem. 39, 1631-1644.
1473 O'Hara, G.W., Daniel, R.M., 1985. Rhizobial denitrification: a review. Soil Biol. Biochem. 17, 1-9.
1474 Okubo, T., Ikeda, S., Kaneko, T., Eda, S., Mitsui, H., Sato, S., 2009. Nodulation-dependent
1475 communities of culturable bacterial endophytes from stems of field-grown soybeans. Microbes
1476 Environment. 24, 253–258.
1477 Palmqvist, K., Dahlman, L., 2006. Responses of the green algal foliose lichen *Platismatia glauca* to
1478 increased nitrogen supply. New Phytol. 171(2), 343-56.

1479 Pappa, V.A., Rees, R.M., Walker, R.L., Baddeley, J.A., Watson, C.A., 2011. Intercropping reduces
1480 nitrous oxide emissions and leaching from an arable rotation. Agric. Ecosyst. Environ. 141(1-2),
1481 153-161.
1482 Paterson, E., 2003. Importance of rhizodeposition in the coupling of plant and microbial
1483 productivity. Eur. J. Soil Sci. 54, 741-750.
1484 Paterson, E., Gebbing, T., Abel, C., Sim, A., Telfer, G., 2007. Rhizodeposition shapes rhizosphere
1485 microbial community structure in organic soil. New Phytol. 173, 600-610.
1486 Philippot, L., Piutti, S., Martin-Laurent, F., Hallet, S., Germon, J.C., 2002. Molecular analysis of the
1487 nitrate- reducing community from unplanted and maize-planted soils. Appl. Environ. Microbiol.
1488 68, 6121-6128.
1489 Powlson, D.S., Brookes, P.C., Christensen, B.T., 1987. Measurement of soil microbial biomass
1490 provides an early indication of changes in total soil organic-matter due to straw incorporation.
1491 Soil Biol. Biochem. 19, 159-164.
1492 Rademaker, J.L.W., Louws, F.J., Rossbach, U., Vinuesa, P., De Bruijn, F. J., 1999. Computer-
1493 assisted pattern analysis of molecular fingerprints and database construction. In: Akkermans A.
1494 D. L., van Elsas J. D., de Bruijn F. J. (Eds.), Molecular Microbial Ecology Manual, 1–33 sect.
1495 7.1.3. Kluwer Academic Publishers, Dordrecht, NL.
1496 Riffaldi, R., Saviozzi, A., Levi-Minzi, R., 1996. Carbon mineralization kinetics as influenced by
1497 soil properties. Biol. Fertil. Soils. 22, 293-298.
1498 Rochette, P., Janzen, H., 2005. Towards a revised coefficient for estimating N_2O emissions from
1499 legumes. Nutr. Cycl. Agroecosys. 73, 171-179.
1500 Scalise, A., Tortorella, D., Pristeri, A. Petrovičová, B., Gelsomino, A., Lindrström, K., Monti, M.,
1501 2015. Legume-barley intercropping stimulates soil N supply and crop yield in the succeeding
1502 durum wheat in a rotation under rainfed conditions. Soil Biol. Biochem. 89, 150-161.
1503 Scott, A., Chrichton, I., Ball, B.C., 1999. Long-term monitoring of soil gas emissions with closed
1504 chambers using automated and manual systems. J. Environ. Qual. 28, 1637-1643.

Sey,B.K., Manceur,A.M., Whalen,J.K., Gregorich,E.G. & Rochette,P. 2010. Root-derived respiration and nitrous oxide production as affected by crop phenology and nitrogen fertilization. Plant and Soil, 326, 369-379.

Silveira, M.L.A., 2005. Dissolved organic carbon and bioavailability of N and P as indicators of soil quality. Sci. Agric. 62, 502-508.

Smith, K.A., Clayton, H., McTaggart, I.P., Arah, J.R.M., Scott, A., 1995. The measurements of nitrous oxide from soil using chambers. Philos. Trans. R. Soc. London, Series B. 351, 327-338.

Sparling, G.P., 1981. Microcalorimetry and other methods to assess biomass and activity in soil. Soil Biol. Biochem. 13(2), 93-98.

Sparks, D.L., 1996. Methods of Soil Analysis, Part 3 - Chemical Methods. SSSA Book Series No. 5. SSSA-ASA, Madison, WI.

Tortorella D., Gelsomino A., 2011. Influence of compost amendment and maize root system on soil CO_2 efflux: a mesocosm approach. Agrochimica. 3, 161-177.

Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil microbial biomass C. Soil Biol. Biochem. 19, 703-707.

Watson, C.A., Baddeley, J.A., Edwards, A.C., Rees, R.M., Walker, R.L., Topp, C.F.E., 2011. Influence of ley duration on the yield and quality of the subsequent cereal crop (spring oats) in an organically managed long-term crop rotation experiment. Org. Agric. 1, 147-159.

Table 1 – Chamber growth conditions during the 97-day experimental period were in accordance to the 26-year average climatic data recorded between April and August in a Mediterranean environment (Reggio Calabria, Southern Italy). The relative humidity was kept stable at 70%. Lighting was produced by cool white fluorescent bulbs at an average intensity of 1160 lux.

Growth period (day)	Temperature (°C)		Photoperiod (h) Day/Night
	Day	Night	
0-20	18.2 ± 0.3	10.2 ± 0.3	6.5/17.5
21-40	23.4 ± 0.3	14.6 ± 0.2	8/16
41-60	28.0 ± 0.3	18.9 ± 0.3	9.5/14.5
61-80	30.6 ± 0.3	21.7 ± 0.2	10.5/13.5
81-97	31.2 ± 0.4	22.5 ± 0.2	9.5/14.5

Table 2 – Soil pH, EC, organic C and total N in the soil (mean ± SD, *n = 3*) was measured at the beginning and at the end of the experimental period. Symbols – and + represent absence or presence of amendment in soils. For each sampling time, different letters in the columns indicate significant differences among treatments (Tukey's HSD test at $P < 0.05$). Significant effects due to treatment, amendment, time and their interactions on the variability of data (*F*-value from three-way ANOVA, treatment x amendment x time, with corresponding *P* values[a]) are also shown at the bottom.

	Treatment		pH	$EC_{1:2}$ (dS m^{-1})	C_{org} (mg g^{-1})	N_t (mg g^{-1})
Pre-sowing	Nitouche	-	6.19 ± 0.06	0.10 ± 0.01	34.13 ± 1.75	2.50 ± 0.10
		+	6.24 ± 0.07	0.11 ± 0.01	29.94 ± 5.34	2.33 ± 0.29
	Zero4	-	6.17 ± 0.04	0.11 ± 0.01	34.13 ± 1.75	2.50 ± 0.10
		+	6.29 ± 0.03	0.11 ± 0.01	28.97 ± 4.41	2.33 ± 0.28
	Triticale	-	6.21 ± 0.04	0.10 ± 0.01	34.87 ± 0.92	2.53 ± 0.06
		+	6.23 ± 0.06	0.11 ± 0.01	30.07 ± 5.48	2.50 ± 0.20
	Triticale/Nitouche	-	6.18 ± 0.04	0.10 ± 0.01	34.40 ± 0.26	2.53 ± 0.06
		+	6.26 ± 0.08	0.11 ± 0.01	33.73 ± 1.60	2.60 ± 0.10
	Triticale/Zero4	-	6.19 ± 0.06	0.10 ± 0.01	33.83 ± 1.24	2.50 ± 0.10
		+	6.24 ± 0.07	0.11 ± 0.01	33.47 ± 2.06	2.60 ± 0.11
	Bare soil	-	6.21 ± 0.04	0.11 ± 0.01	34.27 ± 1.76	2.53 ± 0.12
		+	6.28 ± 0.03	0.11 ± 0.01	29.97 ± 5.58	2.50 ± 0.10
Harvest	Nitouche	-	6.31 ± 0.04 [a]	0.11 ± 0.01 [b,c]	36.36 ± 4.56	2.15 ± 0.02
		+	6.25 ± 0.03	0.12 ± 0.01 [a,b]	35.03 ± 7.51	2.12 ± 0.15
	Zero4	-	6.24 ± 0.04 [a,b]	0.10 ± 0.01 [c]	32.19 ± 0.92	2.06 ± 0.03
		+	6.27 ± 0.04	0.12 ± 0.02 [b]	37.54 ± 3.51	2.07 ± 0.01
	Triticale	-	6.18 ± 0.03 [a,b]	0.13 ± 0.01 [a]	30.32 ± 1.37	2.08 ± 0.06
		+	6.31 ± 0.03	0.10 ± 0.01 [b]	33.62 ± 2.31	2.05 ± 0.06
	Triticale/Nitouche	-	6.21 ± 0.03 [a,b]	0.12 ± 0.01 [a,b]	36.96 ± 1.52	2.09 ± 0.10
		+	6.30 ± 0.03	0.11 ± 0.01 [b]	28.12 ± 2.12	1.87 ± 0.09
	Triticale/Zero4	-	6.21 ± 0.02 [a,b]	0.12 ± 0.01 [a,b]	34.62 ± 1.06	2.10 ± 0.01
		+	6.28 ± 0.07	0.10 ± 0.01 [b]	30.58 ± 2.05	1.96 ± 0.19

Bare soil	-	6.16 ± 0.01 [b]	0.10 ± 0.01 [c]	30.98 ± 0.43	2.09 ± 0.07
	+	6.29 ± 0.05	0.15 ± 0.01 [a]	28.89 ± 1.30	1.98 ± 0.32
Factor	**df**				
Treatment (T)	5	1.866 ^{ns}	1.866 ^{ns}	1.172 ^{ns}	0.510 ^{ns}
Amendment (A)	1	67.903 ***	67.903 ***	5.815 *	2.276 ^{ns}
Time (Ti)	2	5.026 **	5.026 **	2.238 ^{ns}	121.046 ***
T x A	5	8.130 ***	8.130 ***	0.558 ^{ns}	0.146 ^{ns}
T x Ti	10	1.350 ^{ns}	1.350 ^{ns}	1.571 ^{ns}	1.204 ^{ns}
Ti x A	2	0.500 ^{ns}	0.500 ^{ns}	1.982 ^{ns}	16.213 ***
T x A x Ti	10	3.024 **	3.024 **	2.897 **	1.051 ^{ns}
Error	72				

[a] Levels of significance: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; ns: not significant.

Table 3 – Soil Principal component analysis (PCA) of 16 soil chemical and biochemical variables measured in the six experimental treatments (Nitouche, Zero4, Triticale, Nitouche - Triticale, Zero4 - Triticale, Bare soil as in Materials and Methods) in the unamended soils during the 97-day microcosm experiment. PC loading variables (values ≥ |0.60| are in bold) and percent of total variance explained by the first five factors (eigenvalue >1) are reported. Soil variables are as described in Materials and Methods.

Soil variable	PC1	PC2	PC3	PC4	PC5
PMN	**-0.82**	-0.32	-0.11	-0.06	-0.32
N_t	**-0.82**	-0.24	0.14	0.01	-0.13
R_{bas}	**0.82**	0.15	-0.39	-0.18	-0.23
MBC/C_{org}	**0.80**	-0.35	0.27	0.23	0.14
MBC	**0.79**	-0.34	0.34	0.11	0.14
***q*M**	**0.75**	0.07	-0.54	0.11	-0.15
DOC	**0.73**	0.34	0.28	0.07	0.11
C_0	0.58	-0.07	-0.34	-0.23	**-0.60**
NH_4^+-N	-0.01	**0.83**	0.06	-0.23	-0.19
***q*CO_2**	-0.24	**0.78**	-0.14	0.26	0.17
***q*CO_2/ C_{org}**	-0.20	**0.72**	-0.27	0.37	0.13
MBN	0.37	0.01	**0.70**	0.12	-0.17

NO_3^--N	0.40	-0.45	-0.50	0.10	0.25
C_{org}	-0.01	0.13	0.49	**-0.63**	0.03
pH	0.09	0.15	0.45	**0.60**	-0.52
EC	0.48	0.44	0.17	-0.37	0.12
Variance explained (%)	*33.55*	*17.44*	*13.54*	*8.43*	*6.63*

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1549 **Table 4 –** Principal component analysis (PCA) of 16 soil chemical and biochemical variables

1550 measured in the six experimental treatments (Nitouche, Zero4, Triticale, Nitouche - Triticale, Zero4

1551 - Triticale, Bare soil as in Materials and Methods) in the amended soils during the 97-day

1552 microcosm experiment. PC loading variables (values ≥ |0.60| are in bold) and percent of total

1553 variance explained by the first five factors (eigenvalue >1) are reported. Soil variables are as

1554 described in Materials and Methods.

Soil variable	PC1	PC2	PC3	PC4	PC5
MBC	**0.92**	-0.19	-0.01	-0.04	0.25
MBC/ C_{org}	**0.92**	-0.18	-0.18	-0.05	0.16
***q*CO_2/ C_{org}**	**-0.82**	0.15	0.01	0.18	0.20
***q*CO_2**	**-0.79**	0.17	0.16	0.22	0.23
MBN	**0.77**	-0.27	-0.17	0.18	0.30
N_t	**-0.77**	-0.05	0.11	0.05	0.38
DOC	**0.61**	0.29	0.53	0.02	-0.07
PMN	**-0.61**	-0.28	-0.52	-0.26	0.26
R_{bas}	0.36	**0.83**	-0.22	0.15	0.07
C_0	0.09	**0.78**	-0.32	0.17	0.35
NO_3^--N	-0.01	**-0.71**	-0.18	0.56	-0.13
***q*M**	0.20	**0.66**	-0.59	0.06	-0.13
NH_4^+-N	-0.21	0.53	0.39	-0.20	-0.41
C_{org}	0.18	0.17	**0.68**	0.19	0.56
EC	-0.01	0.06	0.03	**0.91**	-0.28
pH	0.13	-0.07	0.48	-0.06	-0.02
Variance explained (%)	*32.59*	*18.22*	*12.77*	*9.14*	*7.60*

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Table 5 – Average above ground flux emissions (µg ml^{-1}) for the whole experimental period for N_2O, CO_2 and CH_4 followed by carbon dioxide equivalent, expressed in t, and then average below ground flux emissions (µg ml^{-1}) for the whole experimental period for N_2O, CO_2 and CH_4. Symbols – and + represent absence or presence of amendment in soils. Significant effects due to treatment, amendment and their interaction on the variability of soil data (*F*-values from two-way ANOVA, treatment x amendment, with corresponding *P* values[b]) are also shown at the bottom.

Treatment		Above ground			Below ground		
		N_2O	CO_2	CH_4	N_2O	CO_2	CH_4
Nitouche	-	0.39 ± 0.16[b]	1620.34 ± 1022.89	2.10 ± 0.81	0.47 ± 0.04[b]	2805.17 ± 639.15[b]	2.34 ± 0.13
	+	0.38 ± 0.10	2253.45 ± 1608.46	2.10 ± 0.78	0.59 ± 0.12[b]	5075.28 ± 701.74[b]	2.17 ± 0.11[b]
Zero4	-	0.33 ± 0.11[b]	2076.57 ± 1869.64	2.07 ± 0.81	0.66 ± 0.11[b]	4386.84 ± 719.72[b]	2.00 ± 0.14
	+	0.34 ± 0.09	2219.74 ± 1795.18	2.06 ± 0.86	0.72 ± 0.15[b]	7073.73 ± 992.48[b]	2.05 ± 0.14[b]
Triticale	-	0.59 ± 0.55[a,b]	2077.48 ± 1704.66	2.12 ± 0.51	2.08 ± 1.00[b]	3048.84 ± 447.91[b]	2.12 ± 0.15
	+	0.37 ± 0.15	2156.48 ± 1722.16	2.14 ± 0.86	0.56 ± 0.08[b]	10373.01 ± 1115.54[b]	2.26 ± 0.09[b]
Triticale/Nitouche	-	0.86 ± 0.38[a]	1920.04 ± 1616.19	2.35 ± 0.67	2.56 ± 1.17[b]	7799.00 ± 1167.89[b]	2.10 ± 0.14
	+	0.30 ± 0.11	2243.43 ± 1870.47	2.05 ± 0.86	1.30 ± 0.42[a,b]	7714.24 ± 1584.48[b]	2.29 ± 0.09[b]
Triticale/Zero4	-	0.38 ± 0.07[a,b]	2054.57 ± 1878.98	2.11 ± 0.78	0.87 ± 0.33[b]	3725.17 ± 415.45[b]	2.23 ± 0.12
	+	0.36 ± 0.10	2388.89 ± 2103.67	2.07 ± 0.82	0.67 ± 0.14[b]	7743.60 ± 683.45[b]	2.18 ± 0.12[b]
Bare soil	-	0.80 ± 0.33[a,b]	1687.66 ± 1205.27	2.17 ± 0.73	19.70 ± 4.76[a]	7907.47 ± 1193.37[a]	2.31 ± 0.16
	+	0.36 ± 0.08	2389.69 ± 2266.00	2.14 ± 0.89	1.95 ± 0.37[a]	17480.05 ± 1398.95[a]	3.28 ± 0.37[a]
Factor	**df**						
Treatment (T)	**5**	3.286 **	0.050 ns	0.062 ns	12.008 ***	14.550 ***	4.173 **
Amendment (A)	**1**	18.408 ***	1.064 ns	0.141 ns	13.033 **	44.209 ***	3.216 ns
T x A	**5**	4.116 **	0.083 ns	0.093 ns	9.221 ***	5.023 ***	2.374 *
Error	**84**						

[a] Different letters in a column indicate significant differences among treatments (Tukey's test at $P < 0.05$). [b] Levels of significance: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; ns: not significant.

Table 6 – Emission intensities (total cumulative N_2O measurements divided by the total biomass for the whole experimental period), expressed in g per t of total biomass. Symbols – and + represent absence or presence of amendment in soils. Significant effects due to treatment, amendment and their interaction on the variability of soil data (*F*-values from two-way ANOVA, treatment x amendment, with corresponding *P* values[b]) are also shown.

Treatment		Intensities		
		N_2O	CO_2	CH_4
Nitouche	-	0.04 ± 0.41	219.30 ± 302.67	171.22 ± 62.25
	+	0.01 ± 0.04	933.45 ± 258.87	143.11 ± 98.77
Zero4	-	0.92 ± 0.33	1066.66 ± 201.18	182.58 ± 153.10
	+	-0.02 ± 0.48	1119.81 ± 647.68	58.53 ± 175.01
Triticale	-	1.33 ± 0.84	898.48 ± 789.79	199.41 ± 310.07
	+	-0.11 ± 0.36	1159.32 ± 256.00	462.84 ± 691.76
Triticale/Nitouche	-	0.68 ± 1.15	774.83 ± 1465.21	166.11 ± 167.84
	+	0.24 ± 0.35	2104.40 ± 1861.08	543.02 ± 879.48
Triticale/Zero4	-	0.18 ± 0.40	846.15 ± 47.80	355.79 ± 44.58
	+	-0.30 ± 0.31	1492.26 ± 1035.41	180.94 ± 409.14
Factor	**df**			
Treatment (T)	**4**	1,754 ^{ns}	0,603 ^{ns}	0,566 ^{ns}
Amendment (A)	**1**	8,884 **	2,008 ^{ns}	0,098 ^{ns}
T x A	**4**	2,463 ^{ns}	0,219 ^{ns}	0,462 ^{ns}
Error	**18**			

[a] Different letters in a column indicate significant differences among treatments (Tukey's test at $P < 0.05$).

[b] Levels of significance: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; ns: not significant.

Figure captions

Fig. 1. Changes in soil dissolved organic C (DOC), basal respiration (R_{bas}), potential mineralisable C (C_0) and microbial biomass C (MBC) (mean ± SD, *n=3*) in unamended (left) and amended (right) microcosm soils at three sampling times (0, 62 and 97 DAS) over the 97-day experimental period for the six treatments: Nitouche, Zero4, Triticale, Triticale-Nitouche, Triticale-Zero4, bare soil.

Fig. 2. Changes in KCl-extractable ammonium-N (NH_4^+-N), KCl-extractable nitrate-N (NO_3^--N), potential mineralisable N (PMN) and microbial biomass N (MBN) (mean ± SD, *n=3*) in unamended (left) and amended (right) microcosm soils at three sampling times (0, 62 and 97 DAS) over the 97-day experimental period. Treatments are as in Fig. 1.

Fig. 3. Changes in mineralization coefficient (*q*M), metabolic quotient (qCO_2), qCO_2/C_{org} ratio and microbial quotient (MBC/C_{org}) (mean ± SD, *n=3*) in unamended (left) and amended (right) microcosm soils at three sampling times (0, 62 and 97 DAS) over the 97-day experimental period. Treatments are as in Fig. 1.

Fig. 4. PCA ordination biplot (PC1 *vs* PC2) of 16 soil chemical and biochemical variables (loadings, see Materials and Methods) measured in the six experimental treatments (Nitouche, Zero4, Triticale, Triticale-Nitouche, Triticale-Zero4, bare soil as in Materials and Methods) (scores) at three sampling times (pre-sowing, flowering, harvest) in the unamended (A) and the amended soils (B) during the 97-day microcosm experiment. The biplot has the same origin for scores and loadings.

Fig. 5. Hierarchical classification (Pearson's similarity coefficient, Ward's clustering method) of banding patterns generated by ARISA of PCR-amplified 16S rRNA gene-coding fragments from soil-extracted bacterial DNA from no-residue (A) and residue (B) added microcosms at

1594 two sampling times (62 and 97 DAS) over the 97-day experimental period. Treatments are as
1595 in Fig. 1. Each bar averages three microcosm replicates. Scale bar (0–100) indicates the
1596 similarity level.
1597

Figure 1 (C pools)

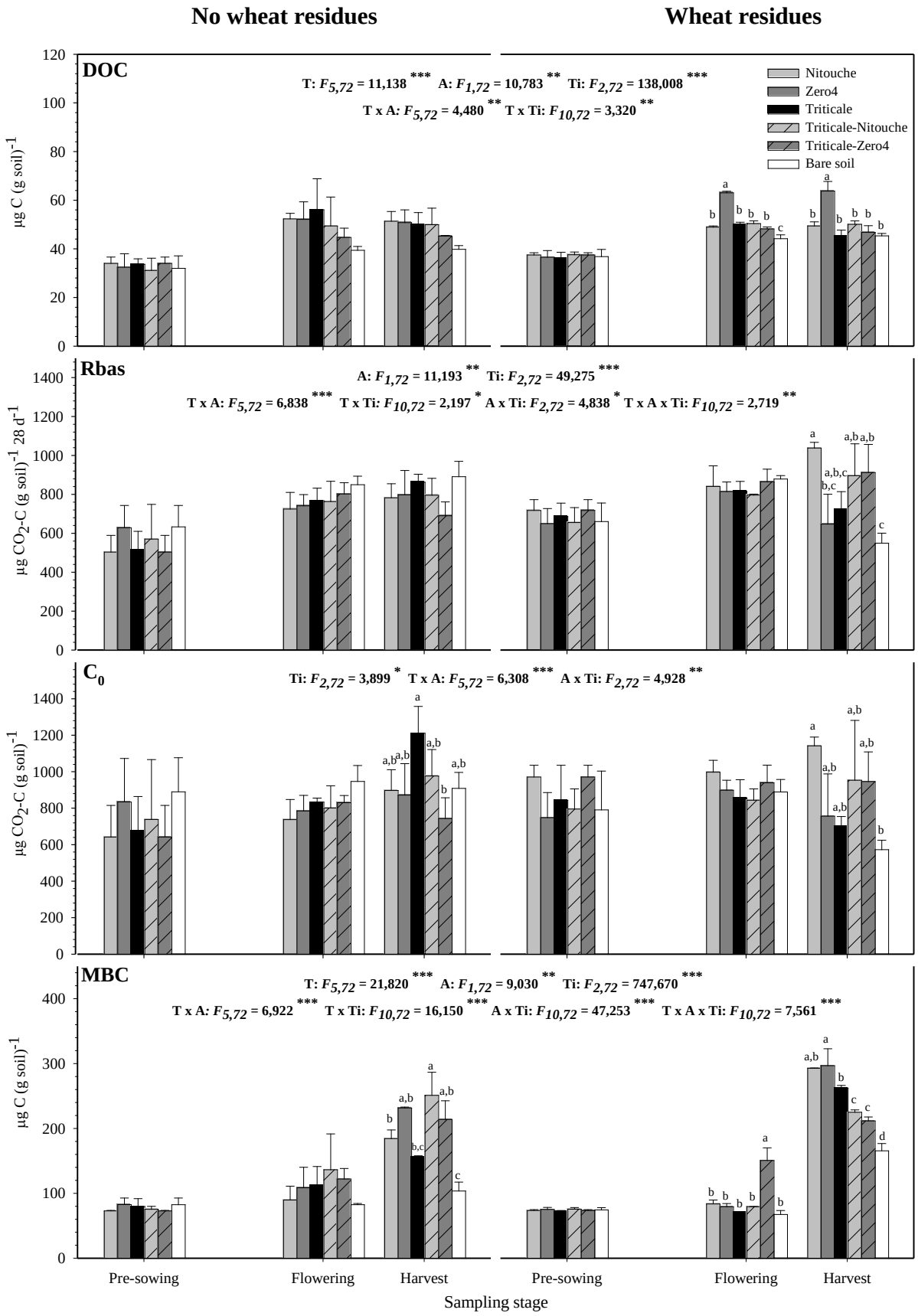
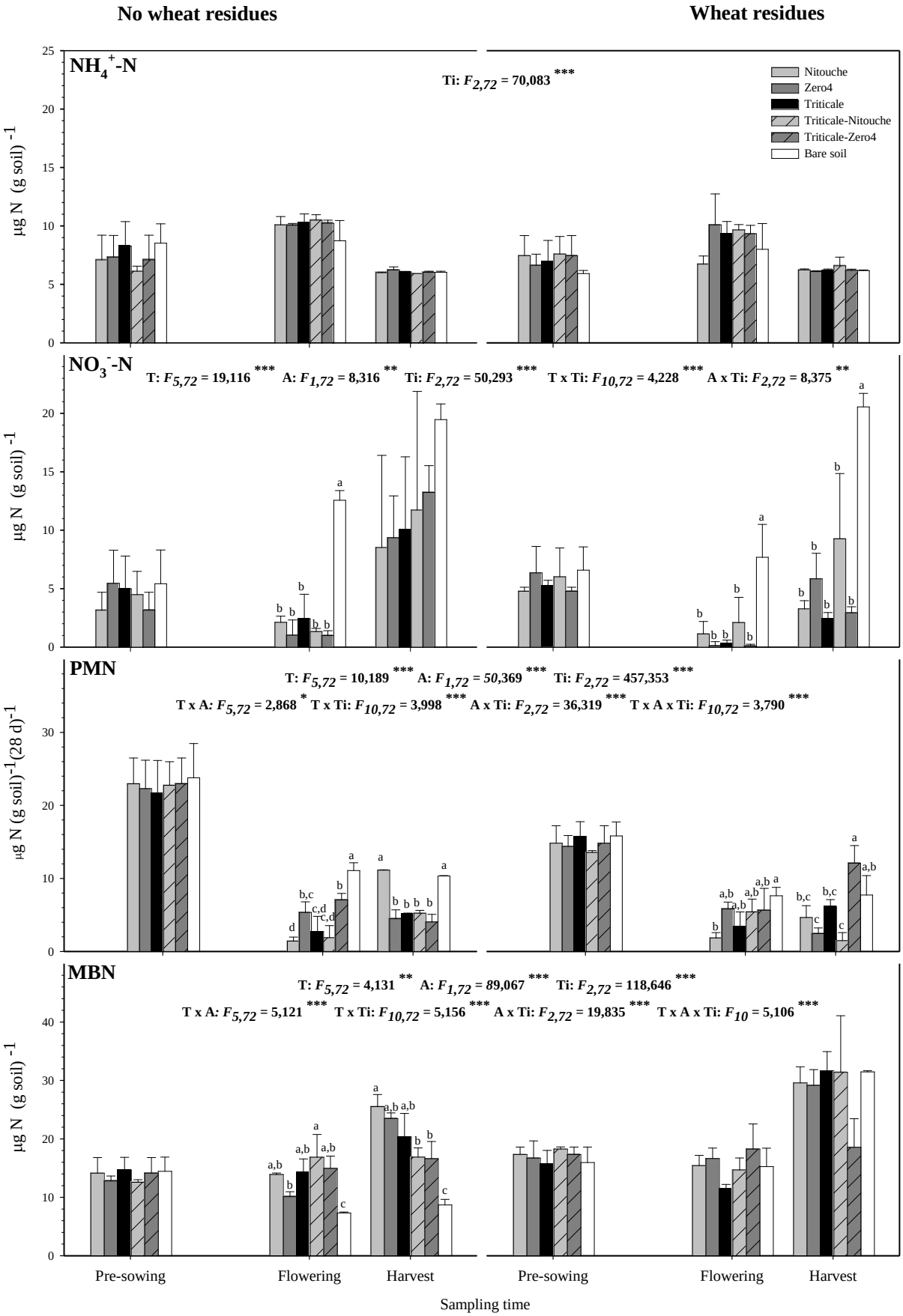
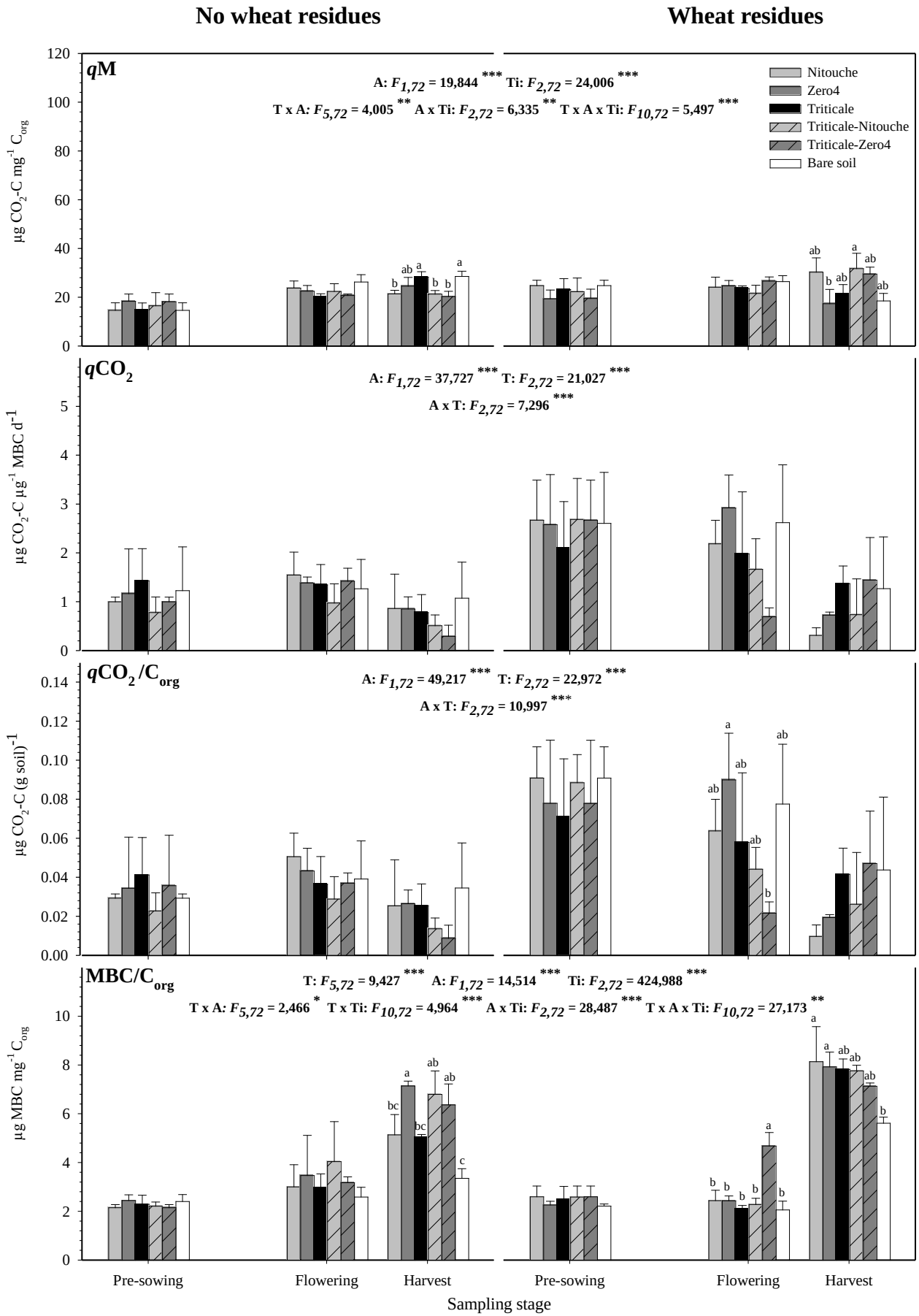


Figure 2 (N pools)



1603 **Figure 3 (Ecophysiological indices)**



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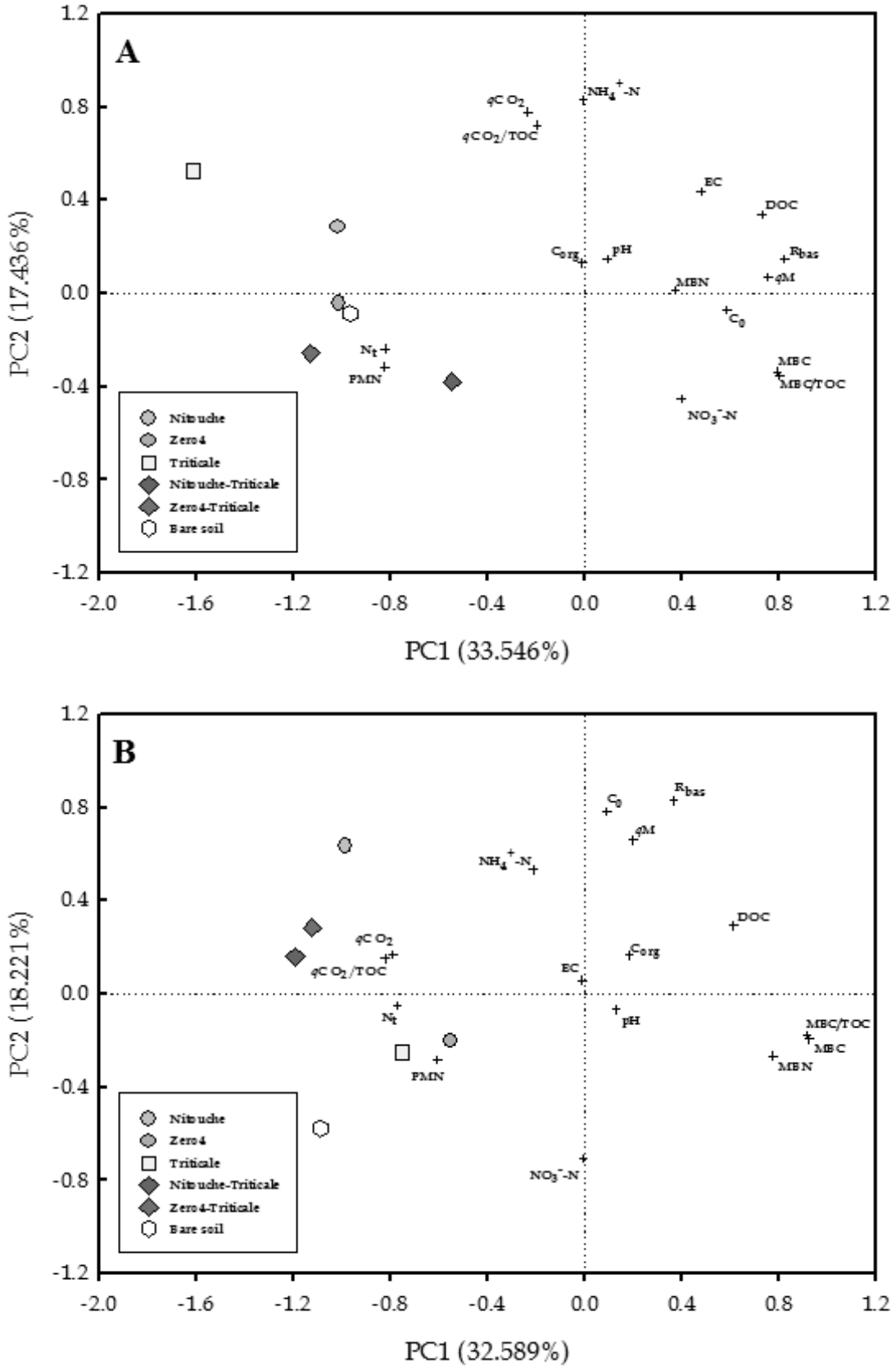
1606 **Figure 4 (PCA analysis)**



1607

Figure 5 (ARISA analysis)